

Methicillin-Resistant *Staphylococcus aureus* (MRSA) in Food Production Animals

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Summary

Until recently, reports on MRSA in livestock were mainly limited to occasional detections in dairy cattle mastitis. Since 2005 however, studies show the existence of an MRSA clone, CC398, which has been reported colonizing pigs, veal calves and broiler chickens and infecting dairy cows. Many aspects of its prevalence in pigs are not yet understood. In other livestock, colonizing capacity and reservoir status are still unclear.. MRSA CC398 has also been detected in meat but, like for other MRSA, the risk this might pose is rather unclear. For now, the most worrying aspect of MRSA CC398 seems to be its capacity to spread to humans. This might complicate MRSA control measures in human healthcare, posing for urgent research into risk factors and transmission routes. Overall, infections with MRSA CC398 are much less reported than carriage. More investigation into its pathogenic potential is though required. Also the origin and evolution of this clone remain to be revealed.

Introduction

The European conference on MRSA, organised last year by the Federation of Veterinarians of Europe, concluded that it was time to tackle MRSA (1). Ten years before, a conference on MRSA organised by veterinarians would have seemed rather peculiar. However, the ever emerging recognition of a new type of MRSA, believed to be of animal origin, and the expanding number of reports on transfer of MRSA between animals and humans, has led to the growing awareness that MRSA is now a problem in both human and veterinary medicine. Looking into the epidemiological aspects of animal MRSA, food production animals (cattle, pigs and poultry, further referred to as livestock) are of particular concern. Not only are they recorded as the primary source of the newly emerging MRSA type, studies also suggest that they are involved in transfer of MRSA strains between animals and humans (and vice versa). Indeed, as livestock they are in close contact with part of the human population (farmers, farm co-workers, veterinarians etc.) on the one hand, and on the other, once they have actually entered the food chain, they might serve as convenient vehicles for bacterial transfer, possibly threatening food handlers and consumers.

In this review, the current knowledge on the prevalence, epidemiology, evolution and medical importance of MRSA in both livestock and derived food products is summarized. As an introduction, the most relevant facts on *S. aureus* and methicillin resistance are listed, followed by a short description of the most frequently used typing methods for both methicillin-susceptible *S. aureus* (MSSA) and MRSA.

MRSA: methicillin-resistant S. aureus

S. aureus is the best characterized species among the staphylococci, a genus of Gram-positive, A-T rich cocci comprising over 50 species and subspecies according to the NCBI Taxonomy browser (2). It forms part of the normal staphylococcal flora of humans and various animal

species (3,4). *S. aureus* is also the most important human pathogenic *Staphylococcus* species, with clinical conditions ranging from common minor skin infections to severe, often life-threatening infections (5).

In animals, *S. aureus* is one of the three major pathogenic *Staphylococcus* species, together with *S. (pseud)intermedius* and *S. hyicus* (6). The scale of infections it may be involved in is as broad as the number of animal species suffering from it, ranging from pneumonia, joint infections, osteomyelitis and septicaemia in poultry (7,8,9), subcutaneous abscesses, mastitis and pododermatitis in rabbits (10,11), dermatitis and cellulitis in horses (12,13) to septicaemia in pigs (4). However, *S. aureus* plays its most significant animal pathogenic role as cause of intramammary infections in cattle and small ruminants (6,14,15,16), leading to considerable economic losses in cattle farming (6,17,18).

S. aureus owes its strong pathogenic capacities to the presence of a large number of various virulence factors (5,19,20,21,22,23,24). In addition, an important impediment in the control of *S. aureus* infections is its tendency to gain resistance to almost all classes of antimicrobial agents which it is subjected to (25). Of particular concern is the acquired resistance to the β -lactamase stable β -lactam antibiotics, historically known as methicillin resistance. Indeed, methicillin resistance is caused by the expression of an alternative penicillin-binding protein, called PBP2a or PBP2' (26,27). Since PBP2a shows a very low affinity for almost all β -lactam antibiotics (27), methicillin-resistant *S. aureus* (MRSA) is resistant to almost all antibiotics of this very comprehensive group, of which many members are still widely used in both human and veterinary medicine.

PBP2a is encoded by the *mecA* gene (28,29). This gene is localized in a mobile genetic element, named the Staphylococcal Cassette Chromosome *mec* (SCC*mec*). According to their structural composition, SCC*mec* elements are categorized into different types. Each type is marked with a capital number. While initially it seemed that there were only a few different

SCC*mec*-types, it has become clear that more exist, and the nomenclature is rapidly evolving. For the moment, eight different types are recognized (Table 1). These types are all based on SCC*mec*-elements found in human MRSA strains.

Molecular typing of (MR)SA

PFGE

Pulsed-Field Gel Electrophoresis, assigning isolates into pulsotypes, is presently considered as the gold standard method for typing of *S. aureus*, of both human and animal origin. As a standard for *S. aureus*, the enzyme *Sma*I is used for the macro restriction (36,37,38).

MLST

In MultiLocus Sequence Typing of *S. aureus*, internal fragments of seven housekeeping genes are amplified and sequenced (39,40). Via the *S. aureus* MLST database (41), a sequence type (ST) is assigned (40). Strains that differ in only one or two loci are called Single Locus Variants (SLVs) and Double Locus Variants (DLVs), respectively. With 'Based Upon Related Sequence Types' (BURST) analysis, STs, SLVs and DLVs are grouped into Clonal Complexes (CCs). In a CC, the ST that has the highest number of different SLVs and DLVs is called the ancestral ST, and the CC is numbered after its ancestral ST (42).

***spa*-typing**

The polymorphic X-region of the staphylococcal protein A (*spa*-) gene contains a variable number of different repeats of mostly 24-bp (43). In *spa*-typing, this repeat region is amplified and sequenced. The total number of repeats and the sequence of each repeat determine a repeat profile, the *spa*-type (44,45).

Recently, the Panel on Biological Hazards of the European Food Safety Authority (EFSA) has recommended *spa*-typing for discrimination between MRSA strains from livestock (46). It

should be noted that most of these strains are not typable with PFGE following *Sma*I digestion and that they generally belong to the ST398 sequence type (see below).

SCC*mec*-typing

An SCC*mec*-element is composed of two essential gene complexes: the *mec*-complex, containing *mecA* and its direct regulatory genes, and the *ccr*-complex, responsible for the mobility of SCC*mec* (30,47,48,49,50). According to their structural composition, variants of these complexes are distinguished (51). The assignment of SCC*mec*-types is essentially based on which variant of each complex is present (Table 1). This is mostly investigated using PCR techniques. Both simplex PCRs (49,52) and multiplex PCRs (53,54,55,56) have been developed.

While the *mec*- and *ccr*-complexes show some limited variation, the other parts of SCC*mec*, called J-regions, can vary greatly within and between SCC*mec*-types (51). Based upon differences in the J-regions, subtypes of SCC*mec* are distinguished (e.g. 57,58,59), and these can also be detected by PCR (53,54,55,60).

An important impediment of the abovementioned methods is that they are all based on SCC*mec*-sequences found in MRSA strains from human origin. Recent studies have shown that these methods fail to identify some SCC*mec*-elements found in MRSA strains from livestock origin (61,62) and many SCC*mec*-elements found in methicillin-resistant non *S. aureus* staphylococci (MRNaS) (63,64). As it can thus be expected that additional SCC*mec*-types will be described in the near future, the present methods will need continuous updating. Indeed, the recently identified SCC*mec*-types VII and VIII for example (34,35) are not included in the current methods.

History of MRSA

The history of MRSA is mainly situated in human medicine and started in 1961, when MRSA was first isolated in a UK hospital (65,66). From then onwards, MRSA began to spread in hospitals all around the world, but at the end of the 1980's and during the 1990's its prevalence truly exploded in many countries (67,68,69,70). In the beginning of the present century, it was shown that the majority of the at that time known international epidemic hospital strains, named Hospital-Acquired MRSA (HA-MRSA), belonged to only five CCs: CC5, CC8, CC22, CC30 and CC45 (42), and that they generally possessed one of the larger *SCCmec*-types I-III (71), partly explaining their resistance to most clinically used antimicrobial agents (5,72,73). As it is assumed that in humans, the use of large quantities of antimicrobial agents can lead to selection and emergence of organisms resistant to these agents (74), prolonged antimicrobial therapy has been designated a risk factor for the acquisition of HA-MRSA (75,76), as have prolonged hospitalization, care in an intensive care unit, surgical procedures, and close proximity to a patient in the hospital who is infected or colonized with MRSA (77,78).

While the problems with HA-MRSA were not at their full width yet, a second phase in the history of MRSA dawned halfway the 1990s, when MRSA infections involving strains different from HA-MRSA were increasingly documented in non-hospitalized patients (79,80,81,82,83). Such cases, called Community-Associated or Community-Acquired MRSA (CA-MRSA), have since been reported worldwide. Although there has been some discussion about the origin of several (early) cases (84), analysis of the genetic background of CA-MRSA strains has shown a clear distinction from typical HA-MRSA, as they predominantly belong to ST1, ST8, ST30, ST59, ST80 and ST93 (85). In addition, CA-MRSA mostly possess the smaller *SCCmec*-types IV and V (32,52,86), which is assumed to be at least partly explanative for the generally more antimicrobial susceptible phenotype of CA-MRSA. The carriage of the genes encoding Panton-Valentine leukocidin (PVL), a cytotoxin believed by

many authors to be responsible for severe infections of the skin and soft tissues (90,91) and highly lethal necrotizing pneumonia (92,93,94), is considered to be typical for certain CA-MRSA strains (85,87). The pathogenic role of PVL is though still under discussion (88,89). Compared to HA-MRSA, CA-MRSA also seems to possess different risk factors for acquisition, as it has been most often reported in populations of intravenous drug users, men who have sex with men, prison inmates, contact sport teams, military recruits and children (80,95,96,97,98,99,100,101).

Although MRSA was first isolated in animals already in 1972, from Belgian cows with mastitis (102), animals seemed not to play a role of significance during most part of the history of MRSA. Based on the results of at that time available biotyping methods, it was concluded that those first isolates were from human origin (103). Also in occasional later reports on MRSA isolated from animals (mainly companion animals) the strains were mostly human genotypes (104,105,106,107,108). This seemed not surprising, seen the increasing prevalence of MRSA in non-hospitalized community members at the time. Although since, the problem of (human) MRSA in companion animals has rather expanded, whole new concerns raised in 2005, when MRSA was found associated with pig farming in the Netherlands (109).

This third phase in the history of MRSA was initiated 'accidentally', by the unexpected isolation of MRSA from a family of pig farmers and one of their pigs (109). Results of subsequent investigations showed that pig farmers from the same geographical region were carrying MRSA in a >760 x higher carriage rate than the general Dutch population..

Remarkably, PFGE analysis of the MRSA strains showed that they were all resistant to digestion with restriction-endonuclease *Sma*I, but *spa* typing and Randomly Amplified Polymorphic DNA (RAPD) analysis proved that all strains were closely related to each other (109). When a few months later a pig farmer from another region together with an unrelated

veterinarian mostly working with pigs, that veterinarian's son and a nurse treating this boy were found also to be colonized with a related MRSA strain, it was concluded that pig farming might pose a significant risk for MRSA carriage in humans (109).

At that time, the importance of specifically this type of MRSA was not clear, and also the extent of the problem concerning farming, of pigs as well as other animals, could only be guessed at. In the following months and years however, a multitude of reports, from the Netherlands as well as many other countries, showed that MRSA of this type not only had spread among pigs, other animal species (veal calves, chickens, horses, dogs, rats) and humans related to farming

(61,110,111,112,113,114,115,116,117,118,119,120,121,122,123,124,125,126), but was also capable of causing disease, in animals (62,115,119,127,128,129) as well as humans (116,119,130,131,132,133,134). It is now understood that this MRSA is disseminating clonally, possessing some typical features:

- non-typability with standard PFGE with *Sma*I-digestion; this was shown to be due to the presence of a new restriction/methylation system leading to protection from *Sma*I digestion (135). It is not clear how this novel system was achieved. The specific system was thus far unknown for *S. aureus* and even for the genus *Staphylococcus* (135).
- being mainly MLST ST398. Very seldom, SLVs or DLVs, like ST752 and ST753 have been reported (116). All strains thus belong to the same CC, CC398 (41,116,130).
- various *spa*-types; the authors have knowledge of 25 different yet related *spa*-types that have been reported to belong to CC398 (Table 2 and Table 3). The majority of these types are combinations of repeat sequences r02, r08, r16, r24, r25 and r34 (Table 2). Some other repeats are present less frequent but are closely related to some of the common repeats (Table 3). As new *spa*-types have continuously been reported so far, it seems likely that more types will be described in the future.

- carriage of *SCCmec*-type IVa or V; some studies also found *SCCmec*-type III (61,116,122). However, the method they used, a multiplex PCR described by Zhang et al. (54), is under debate concerning its reliability for typing *SCCmec*-type III (139). Also, several studies have stated that *SCCmec*-type IV was present (116,123,127,136,140). No subtyping was performed by these authors, so it is not clear whether their strains belonged to IVa. To date, no other subtype than IVa has been found in this MRSA clone.

Remarkably, the *SCCmec*-elements also often appear to be non-typable with the common *SCCmec*-typing techniques (61,62,116).
- general absence of Pantone-Valentine leukocidin (PVL), differentiating these strains from typical CA-MRSA strains. Also many other virulence(-associated) factors, known to be present in typical HA- or CA-MRSA strains, have been shown to be absent in MRSA of ST398 (141,142). Despite this large absence of virulence(-associated) factors, MRSA ST398 strains have been found causing disease, in both animals (62,115,119,127,128,129) and humans (116,119,130,131,132,133). It is as yet unclear which factors are involved. More investigations are urgently needed to elucidate this.
- resistance against tetracycline, and frequent resistance against macrolides, lincosamides, aminoglycosides and trimethoprim. Also fluoroquinolone-resistance has been reported (61,62,112,119,123,127,132).

Some different descriptions have been proposed for referring to these MRSA strains. Because of their typical resistance to *Sma*I digestion, they are sometimes called Non-Typable MRSA, NT-MRSA. However, there are arguments against the use of this description. First, it is obvious that these strains are not non-typable. Many other techniques have proven to be useful for typing the so-called NT-MRSA (109,111,143,144). Moreover, when using other restriction enzymes, positive results can be obtained with PFGE (138,144). Second, from bacteraemia blood samples from a Hong Kong hospital collected in 2000-2001, two MRSA

were isolated with a clonal background formerly unknown for Hong Kong, ST398 (145). They however proved to be digestible by *Sma*I, and were assigned to pulsotype I. It seems reasonable that the clonal background should settle the name. ST398 is a type that was virtually absent from the human population before the initial reports in the early 2000s, and typing data seems to support that its recent presence in humans is a direct result of its emergence in livestock, and more specifically, pigs (116,146,147). This supports a livestock origin of these MRSA strains. Consequently, livestock-associated MRSA (LA-MRSA) appears to be the most appropriate description, and this name designation will be used in the further text.

MRSA in livestock

Although LA-MRSA is the most important MRSA clone residing in livestock, it is not the only MRSA type that has been reported in livestock. Below a general overview is given of MRSA in livestock.

Pigs

LA-MRSA seems to be the predominant MRSA strain in pigs, as among numerous recent studies only two mention the detection of non-LA-MRSA in pigs. In Singapore, one ST22-MRSA-IV was isolated from pigs (115). ST22-MRSA-IV had been found before to be increasingly important in the hospital population of Singapore and is also known as UK-EMRSA-15, one of two major hospital clones in the UK (132), indicating human contamination of the pigs. In Canada, 14% MRSA isolates from pigs appeared to belong to the human epidemic CMRSA-2 clone, while 74.4% of the isolates were LA-MRSA (121). The remaining strains belonged to rare clones, not related to LA-MRSA or CMRSA-2 (121).

Most reports on LA-MRSA in pigs come from the Netherlands (111,127,131,136). In Europe, LA-MRSA has further been found in pigs in Germany (119,129), Denmark (112) and Belgium (117,120), while outside Europe, in Canada (121,148), Singapore (115) and the United States (149).

Several studies have described colonization of healthy pigs. At pig level, the reported carriage-rates vary considerably between countries, from 1% in Denmark (112), 18.6% in Canada (121), to approx. 40% in Belgium and the Netherlands (111,117). Farm level rates are generally higher: 66% in Denmark (112), 68% in Belgium (117) and 45% in Canada (121). However, these figures have to be interpreted carefully, as the number of farms included in the studies varied considerably, from 3 in Denmark (112), 20 in Canada (121) to 50 in Belgium (117). In the Netherlands two studies found very different farm level prevalences, 81% positive farms (44 out of 54 farms) (131) compared to 23% positive farms (7 out of 31 farms) (136). Both studies however used different culture techniques. In addition, the result of the former study could have been increased by cross-contamination in the slaughter house, where the sampling was done (131). In the latter study on the other hand, the majority of farms belonged to the ambulatory clinic of the Veterinary Faculty of Utrecht, and in these farms the antimicrobial use is generally more restricted than in other farms, implying a possible underestimation of the farm-level-prevalence (136).

Limited other investigations have been performed to elucidate factors possibly influencing LA-MRSA prevalences in pigs. In one study, different farm management systems showed significant differences in LA-MRSA prevalences, with LA-MRSA being detected in 94% of open farms (fattening farms) compared to 56% in closed farms (farrow-to-finish farms) (117). In another study however, LA-MRSA prevalence seemed to differ greatly when comparing among two closed farm systems; one production system was highly MRSA positive and the other system appeared MRSA negative (149). As a cause for this, differences in other aspects,

such as breed and herd size, were suggested by the authors (149). A third factor they suggested was the origin of the sows. The sow herds of both production systems had been repopulated shortly before the date of sampling. As it was, part of the sows of the MRSA positive system had been imported from Canada, where pigs have been found to be affected by LA-MRSA (121). The sows from the other system came from Michigan, U.S. (149). Although the authors could not give epidemiological evidence, LA-MRSA was thus possibly brought into the positive farm via import of affected live swine or pork products. This however should not mean Canada is the origin of LA-MRSA in the U.S., as at that time the presence of LA-MRSA in other regions of the U.S., such as Michigan, had possibly not been investigated.

Although certainly more studies are required to reliably assess the influence of farm management and related aspects on LA-MRSA prevalence, these studies suggest an important role for national and international pig trading in the dissemination of LA-MRSA in pig farming. This was also suggested by a Dutch study, in which indications were found that finishing and farrowing farms may get colonized by LA-MRSA through the purchase of colonized pigs from their supplier farms (136). A recent study also showed that piglets from an LA-MRSA positive sow were 1.4 times more likely to be colonized with LA-MRSA than a piglet from a negative sow (148). Purchase of LA-MRSA positive sows will thus facilitate the spread of LA-MRSA in a farm.

Another factor that might be implicated in LA-MRSA prevalence in pigs is age. In a Belgian study, the probability of being MRSA positive was significantly higher for piglets than for both sows and fattening pigs (117). The influence of age was also investigated by a recent Canadian study, in which the dynamics of MRSA colonization in piglets was investigated over time (148). All MRSA appeared to be LA-MRSA and the colonization rates were found to be low initially (below 10%) but increased over time, to 35% positive piglets prior to

weaning and a peak of 64% positive piglets at the age of 42 days. At the last day of sampling (age 70 days) 41% of piglets carried MRSA. However, an earlier Canadian study contrarily found no significant difference in MRSA prevalence between three different age groups of pigs, i.e. suckling pigs, weanlings, and grower/finisher hogs (121). More investigations are thus required to elucidate any possible influence of age.

LA-MRSA has also been isolated, albeit rarely, from infections of pigs. These involved skin infections such as exudative epidermidis (127) or others (115,129) but also infections of the urogenital tract (129) and the uterus and mammary gland (129). For the moment it is unclear which virulence factors are involved. Yet, considering the far higher number of unaffected carriers, LA-MRSA seems to be rather a colonizing strain of pigs.

Cattle

In cows, *S. aureus* plays a significant role as a major cause of mastitis (6) and most studies on MRSA in cattle concern isolation of MRSA from mastitis. Unlike the development in humans, the first detection of MRSA in mastitis (102) was not the beginning of a steady increase in MRSA prevalence. Although more MRSA was detected in some of the originally positive farms on several occasions in the two years after the first isolation (103) and in the subsequent years MRSA was still detected in Belgian routine antibiotic susceptibility tests (150), the prevalence fell and in 1982-1983 MRSA was no longer detected in Belgium, an absence which remained for nearly 25 years (62,150). Also in other countries no MRSA was reported in mastitis for a long time. Reports came more frequently from the early 2000s on. From these reports, it is hard to estimate an overall prevalence of MRSA in mastitis. First, there are often inconsistencies in laboratory methods in different studies, making it difficult to make viable comparisons. The most important example of this is the lack of control on the presence of the *mecA*-gene in many studies, which can result in unreliable data (151), for

example because of the heteroresistance of *Staphylococcus aureus* to methicillin (152).

Several studies reporting MRSA in mastitis not or only partially verified the presence of the *mecA*-gene (153,154,155,156), and should thus be treated with the greatest care when estimating the prevalence of MRSA in mastitis. Also the isolation procedures tend to differ considerably between different studies.

Second, when considering studies in which presence of *mecA* was confirmed (= true MRSA), other essential data can be missing. In France, Alves et al. recently detected true MRSA in *S. aureus* strains collected from mastitic milk and from the nares of cows. They did however not mention from which body site the MRSA strains originated (157).

Third, prevalence of true MRSA in mastitis can be assessed at different levels, i.e. quarter-, cow- and farm-level. Some studies do not provide sufficient data to know on which level their data should be interpreted. For example, a Hungarian study found true MRSA in milk samples from subclinical mastitis originating from only one farm, but it was not elucidated whether all samples originated from different cows or from different quarters. Moreover, it was a longitudinal study, with samples being taken over a two-year period (158). In South Korea true MRSA was found in bovine milk specimens. However, the total number of milk specimens was not given and not all samples originated from mastitis (159). In another report the same author found true MRSA in a specific number of milk samples from different cows but also in this case the number of milk samples originating from mastitis cases was not specified (160).

Only two studies, from South Korea, give adequate information, and they show that the quarter-level prevalence of MRSA in mastitis is very low, ranging from 0.18% (58) to 0.4% (161).

A factor that not so much influences a correct estimation of the MRSA prevalence in mastitis but is important from an epidemiological point of view is the origin of the MRSA strains.

Using biotyping methods the first detection of MRSA in mastitis was found to be presumably of human origin (103). Yet, from the other above mentioned studies, many did not include information or hypotheses on origin of the strains (154,155,156,161). Those that did mostly found a likely human origin (58,158,159,160), although the presence of bovine specific MRSA strains was also suggested (153,157). Recently however, LA-MRSA has been found to be present in Belgian cases of clinical and subclinical mastitis (62). It was shown that nearly 10% of Belgian farms suffering from *S. aureus* mastitis was affected by LA-MRSA and that the farm-level prevalence of LA-MRSA in positive farms varied between 3.9% and 7.4% (62). Also in Germany LA-MRSA has been found in mastitis (141).

As the colonization capacity of LA-MRSA in dairy has not yet been investigated and the current data on LA-MRSA are still sparse, the exact burden of LA-MRSA for dairy cattle farming is not yet clear. Yet, it can be reasonably suspected that the infection of dairy cattle with LA-MRSA will expand in the future. The many species in which it has been found shows that LA-MRSA is relatively unspecific in its host colonisation and might thus very well find a host in cows. In addition, although this has not been substantiated by evidence, the small timescale in which LA-MRSA has attained these different species and has spread to different countries suggests that LA-MRSA can spread easily. As β -lactam antibiotics, β -lactamase-sensitive as well as -stable, are among the most frequently used antibiotics for treatment of mastitis and also tetracyclines, macrolides and aminoglycosides are often included in the treatment or prevention schedule (162,163,164), the typical resistances LA-MRSA exhibits against these antibiotics might cause serious problems for treatment of mastitis. Because risk factors (repeated or enduring contact with a contaminated source) and transmission routes (the milking machine and hands of the farmer) for the spread of normal mastitis-causing *S. aureus* are likely to be the same for spread of LA-MRSA, future research might want to focus on these topics.

While we have found no real evidence that non-LA-MRSA has ever been isolated from another body part of living cattle than the udder, LA-MRSA has been found in the nose of beef calves. In the Netherlands, in one farm 50% of the beef calves appeared to be carrying LA-MRSA in their nose (114). As for dairy cattle, more research is still needed to elucidate whether LA-MRSA has a true reservoir in veal calves.

Poultry

The first report on MRSA in poultry came from South Korea, where MRSA was isolated from chicken arthritis cases. With RAPD typing the MRSA strains appeared highly similar to each other, and they were suggested to share a common ancestor with MRSA strains isolated from humans and bovine milk (160). Three years later three MRSA were reported again in South Korea (159). On the basis of a comparison of the sequence of the *mecI*-gene with human strains, one strain was suspected to be human. The other had a *mecI*-sequence that was previously undetected in humans suggesting these strains were animal specific.

More recent reports concern the detection of LA-MRSA in or associated with poultry. First, a LA-MRSA strain was isolated from chicken droppings in the Netherlands (113). Then, LA-MRSA was detected in Belgian poultry, where LA-MRSA was found in a collection of recent *S. aureus* isolates from nares and cloaca of industrial broilers in Belgium (61). In another Belgian study LA-MRSA was found in broiler chickens but not in laying hens (124). For the moment the reasons for it are unclear. Though, as antibiotics are seldom used in layers, differences in antibiotic use may account for it.

The amount of data is currently too sparse to draw consistent conclusions on LA-MRSA in poultry. It is unclear whether LA-MRSA has an impact on animal and poultry farmer health. More investigations are needed to further elucidate the epidemiology.

MRSA on livestock-derived food products

Besides its importance as hospital and community pathogen, *S. aureus* is also a well-known cause of food intoxication (165,166). *S. aureus* food poisoning is the result of the production of staphylococcal enterotoxins, of which many types have been found in strains of *S. aureus* (166,167). Although these enterotoxins function as superantigens, i.e. they cause immunosuppression and trigger nonspecific proliferation of T-cells, leading to high fever, the clinical outcomes of *S. aureus* food poisoning are mostly relatively mild (165). Therefore, it is estimated that the actual number of foodborne illnesses caused by *S. aureus* is much higher than the reported number (165).

In contrast, MRSA food poisoning is very rare. The only report on MRSA food poisoning comes from the United States, where three adults became mildly ill after they had eaten coleslaw contaminated with an MRSA producing enterotoxin C (168). This strain probably came from a food handler in the market place where the coleslaw was bought and was possibly of hospital origin (168).

MRSA of human origin can also be found on meat. In the Netherlands, an MRSA strain isolated from raw pork appeared upon genotyping to be identical to a well-known human clone, USA300 (ST8-MRSA-IV) (169). Also in the United States MRSA clone USA300 was found on raw pork (170). In that study also another widespread human clone, USA100 (ST5-MRSA-II), was found on raw pork and on a sample of raw beef. Further, in South Korea MRSA of a likely human origin was found twice on chicken meat (160,171). Also in Jordan MRSA of suspected human origin was found on chicken meat (172). All these studies did not report whether the detected strains were capable of producing enterotoxins. In Japan however, an MRSA strain of human origin isolated from raw chicken samples appeared capable of producing enterotoxin C (173).

The aforementioned studies did not elucidate the source of contamination of the meat. The investigated meat was selected from retail shops or meat markets. However, no research was done on presence of MRSA in the people working in these places. There is though one study from Taiwan in which 18 meat market workers were shown to carry MRSA in their nose (174). Yet, in this study no meat was examined.

As humans are capable of contaminating meat, it seems reasonable that human MRSA on meat can also contaminate persons handling or eating contaminated meat. This could be an important route for transmission of MRSA in the community or the hospital. The risk this could pose was illustrated by a Dutch hospital outbreak of MRSA in two hospital units. The outbreak was likely initiated by transmission of MRSA via food contaminated by an MRSA positive healthcare worker involved in food preparation (175). In total, fourteen healthcare workers and 27 patients were attained and of 22 patients that subsequently developed clinical disease, four died. The initial food specimen involved could however not be revealed (175). Indeed, in this regard, it needs to be kept in mind that proving a food specimen was a source of MRSA contamination is likely to be a difficulty in studies investigating this. Per definition, food is sold or bought to eat. Consequently, unless (clinical) results follow immediately upon eating or handling contaminated food specimens, contamination is likely to pass unnoticed initially and by the time it is noticed, if it ever is, the contaminated specimen will often be sold, eaten or thrown away. Hence, contaminated food could hitherto have been much more involved in cases of MRSA colonization or infection than has been reported until now. Thus, even though of the above mentioned studies on human MRSA strains on meat, none reported contamination events due to contact with the contaminated meat, it is perhaps premature to suggest that meat contaminated with human MRSA poses low or no risk for consumers. More investigations, for instance under experimental settings, are needed to gain more insight in the actual risks. Opposite, it also seems unnecessary to make too much commotion about the risks

of contaminated food. Performing the normal hygienic measures when handling foods should suffice until thorough research proves otherwise.

Of special interest in these matters is the emergence and wide spread of LA-MRSA in livestock, which raises the question whether these strains are also present on derived meat and via this way could find an entry key for a larger spread in the human population. A Dutch study proved relatively early that LA-MRSA could be present on pork (169). A very recent and much larger Dutch study confirmed this, and showed in addition a very wide spread of LA-MRSA on many different meat products. In the study, 2217 raw meat products were investigated and MRSA strains were isolated from no less than 264 samples (11.9%). An overwhelming 85% of the strains were LA-MRSA (137). The highest isolation percentages were found in turkey, chicken and veal meat. Not only is this clear proof that LA-MRSA has found its way into the food chain, it is also remarkable because LA-MRSA was present in turkey, lamb and sheep meat while currently no living carriage of LA-MRSA has been reported in these animals. Despite this relatively high number of meat contaminated with LA-MRSA, so far there are no signs that this has contributed significantly to the dissemination of LA-MRSA to humans. This may be at least partly due to the very low numbers in which LA-MRSA was found present on the meat (137). However, the comments concerning the difficulties of proving this, as in the case of human MRSA on meat, also count for LA-MRSA.

In addition to meat, another livestock derived food product that could lead to MRSA food intoxication or serve as vehicle for MRSA transmission is raw milk, when contaminated raw milk is used for the production of cheese. This was reported in Italy, where two MRSA strains of unknown origin were found in dairy cheese products (176). As these strains were found to harbour genes for expression of common staphylococcal enterotoxins, they had the potential to cause food poisoning (176).

Origin and molecular evolution of LA-MRSA

LA-MRSA seems to be primarily associated with pigs. However, since little is known on healthy carriage of *S. aureus* in pigs and the staphylococcal species dominating in infections of pigs is *S. hyicus* (4,6), the emergence of LA-MRSA in pigs was fairly unexpected, and brings up questions on origin and evolution of MRSA ST398.

From MSSA ST398 to MRSA ST398

As both MRSA ST398 and MSSA ST398 have been described, it can be assumed that MRSA ST398 evolved from MSSA ST398 by acquisition of *SCCmec*. Two issues relate to this event: the origin of MSSA ST398 and the circumstances concerning the acquisition of *SCCmec* by MSSA ST398. Unfortunately, very little data are available to elucidate both these issues.

Until now, MSSA ST398 has only been described in humans (41,147,177) and in pigs (112,147), suggesting one of these species is the original host of MSSA ST398. Although it has been more often and way earlier (already in 1997) (41) described in humans than in pigs (first time in 2005) (147), the results of an early French study support the idea that MSSA ST398 is primarily pig-associated. In that study ST398 was found among certain MSSA clones that were prevalent in healthy pig farmers but not in healthy non-farmer controls. As ST398 MSSA strains were also present in infections from pigs, animal to human transmission of this clone was suggested (147). Furthermore, the low number of detections and the relatively late date of the first description in pigs can easily be due to the fact that, following from the low pathogenic relevance of *S. aureus* in pigs, there has been little reason for MSSA (ST398) isolates to be detected in pigs. Nonetheless, it seems that more data are necessary to reliably conclude that MSSA ST398 is essentially pig-specific. Unfortunately, contrarily to

humans, very few databases of *S. aureus* strains isolated from pigs over time are available, making it hard to assess the prevalence of MSSA ST398 in pigs before the recent reports. The issue of SCCmec acquisition by MSSA ST398, evolving then into MRSA ST398, is equally hard to address. In general, little is known to date on the origin of SCCmec and *mecA* and on the epidemiology and mechanisms of SCCmec acquisition. Apart from the very origin of *mecA* in *S. aureus*, which is assumed to have involved a *mecA* homologue present in the coagulase-negative species *Staphylococcus sciuri* (178,179,180,181), it is generally assumed that the presence of SCCmec in a certain MRSA clone has been preceded, somewhere in the evolution of that clone, by a horizontal transfer of SCCmec from another source (71). This source could then be another MRSA strain carrying the specific SCCmec element or, which is often suggested, a methicillin-resistant non *S. aureus* *Staphylococcus* (MRNaS), in which various SCCmec elements are known to be present (182,183,184) and which thus could function as a reservoir for SCCmec. Very little is however known on such transmission events. Although the cassette chromosome recombinase(s) contained in the *ccr*-complex of SCCmec are known to be responsible for integration and excision of the entire SCCmec (31,32), actual transmission events of whole SCCmec elements are very hard to prove, and the few reports on such events largely depend on interpretation of indicative epidemiological and typing data. For example, using such data, a Swedish study recently suggested horizontal transfer of an SCCmec type V between clinical isolates of methicillin-resistant *S. haemolyticus* and MSSA ST45 (185). Yet, without further evidence, the role of MRNaS in the horizontal transfer of SCCmec to MSSA and the frequency of SCCmec transmission events between staphylococcal strains remains merely a case of speculation.

The same accounts for explaining how LA-MRSA evolved from MSSA ST398 strains. With the assumption that MSSA ST398 is originally pig-associated, it can be proposed that SCCmec transmission occurred in pigs, with MRNaS or MRSA as donor species. However,

very little literature is available on presence of MRNaS in pigs, leaving any such donor species to the guess. Also, to date, MRSA strains other than LA-MRSA have rarely been reported in pigs (see above). Nonetheless, as LA-MRSA mostly harbours *SCCmec*-types IVa and V and the smaller *SCCmec* types IV and V are considered to be typically carried by CA-MRSA, human CA-MRSA strains could very well have been the donor species. Indeed, human MRSA strains could be present more frequently in pigs, because, similarly to MSSA in pigs, the availability of few reports could be due to a lack of studies investigating MRSA in pigs. However, an interesting idea is that the acquisition of *SCCmec* by ST398 MSSA did not occur in pigs but in humans. This would agree with the earlier detection of ST398 MRSA in humans than in pigs. Indeed, before the first report on LA-MRSA in pigs (109), ST398 MRSA was detected in a Dutch woman (41,131) and in a French pig farmer (147). A possible course of events could thus have been that after transfer to one or more humans, most likely farmers, an ST398 MSSA strain from pigs acquired *SCCmec* from a CA-MRSA strain, and after recolonization of one or more pigs, such ST398 MRSA strain started to spread among other pigs. That the first detections of MRSA ST398 occurred in the Netherlands and France does not necessarily imply that these events took place in one of those countries. Especially the Netherlands seem an unlikely country, as the MRSA prevalence in both the hospital and the community is one of the lowest of the world.

Regardless of the exact facts of these matters, the fact that multiple *SCCmec*-types have been identified in LA-MRSA indicates that LA-MRSA must have arisen on multiple occasions from MSSA ST398 strains. This brings along other questions. It is for example not clear whether the two most frequently detected types, IVa and V, were already present from the very beginning, or whether one element was responsible for the first cases and the other was acquired later on. The very first reports of ST398 MRSA did not include information on the *SCCmec*-type present (41,109,131,147). Moreover, several untypable *SCCmec* elements have

been found in LA-MRSA but it is unknown exactly how many different elements LA-MRSA carries. In addition, since LA-MRSA has been detected in a large geographical area and in many different species, the acquisition of *SCCmec* could have occurred in different species and different countries. However, until now ST398 MSSA has only been reported from the Cape Verde islands (41), France (147), the United States and the Dominican Republic (177) and was found only in humans (41,147,177) and pigs (147).

In conclusion, the origin and evolution of LA-MRSA following acquisition of different *SCCmec* elements by different MSSA ST398 strains involves many unanswered questions, which for the moment can only be addressed with speculations. The lack of long-term data on the presence of *S. aureus* in pigs - MSSA as well as MRSA and human as well as non-human strains - makes it reasonable to fear many of these questions will remain unanswered.

Acquisition of Panton-Valentine leukocidin

In addition to the *SCCmec* issues, another event that deserves consideration when trying to unravel the molecular evolution of LA-MRSA is the acquisition by certain strains of the genes encoding the Panton-Valentine leukocidin (PVL). Strains of CC398 MRSA that did possess the PVL genes have been found causing human infections in China (140) and Sweden (186). However, the large majority of CC398 MRSA strains that have been investigated appeared not to possess these genes, even when the strains originated from infections (e.g. 116,119,130,134,149,187).

It is not clear how the aforementioned LA-MRSA strains acquired PVL. The genes encoding PVL are carried on mobile genetic elements (MGEs) (188). As both cases reported no link between the patients and animal contact but instead found medical histories of the patients that are typical for HA-MRSA (140) and CA-MRSA (140,186), perhaps it concerned LA-

MRSA strains that resided already for a longer period in the hospital or the community environment and acquired PVL from human MRSA strains.

Despite the fact that the importance of PVL as virulence factor is still controversial (88,89,91,94), these cases illustrate the potential of LA-MRSA to take up virulence factors on MGEs. A further spread of such MGEs in LA-MRSA may impose a serious risk for both human and animal health, seen the wide spread of LA-MRSA in some animals and its potential to colonize and infect humans.

LA-MRSA in humans

Since the medical significance of LA-MRSA for veterinary medicine is currently rather low, perhaps the most worrying aspect of LA-MRSA is its apparent capacity to transfer between its animal carriers and people in close contact with them. This has been extensively shown in pig farming. Several studies, from many different countries, found that living or working on a farm with colonized pigs were risk factors for LA-MRSA carriage (109,116,121,123,132,133,149). In the Netherlands, an increased risk of LA-MRSA carriage has also been shown in veal calf farming (114,116,189,190), even though actual carriage of LA-MRSA by veal calves has been reported only once (114). An increased risk has not yet been shown for dairy or meat cattle farmers. To a lesser extent, also poultry farmers have been found colonized as a result of LA-MRSA carriage by their animals (113). In addition, also veterinarians working with pigs and cattle were found to be at higher risk for carriage of LA-MRSA (109,110,116,122,123).

Despite the fact that its transferring capacity was pretty clear from the first reports on LA-MRSA (109,131), until today, many features concerning it remain unclear. For example, as such an extensive transfer between humans and animals has not been reported for any other clone of MRSA, it can be assumed that LA-MRSA possesses special mechanisms or

characteristics. However, this assumption has not yet been verified, and consequently, possible mechanisms have not yet been elucidated. Furthermore, only little information is available on actual risk factors and associated transmission routes. Equal to other HA-MRSA and CA-MRSA, the most obvious risk factor and route of transmission of LA-MRSA is direct contact with colonized patients, i.e. animals. This has been proven in pig and veal calf farming (116,190). Yet, though it can be assumed to be important also in other animal farming activities, so far this has not been substantiated with evidence. Further, the duration of animal contact and the percentage of MRSA positive animals were shown to be risk factors in veal calf farming. In addition, contact with a contaminated environment has been suggested (174). Such environment could also include the air, as studies have shown that air in pig and cow stables may contain considerable amounts of (antimicrobial resistant) bacteria (191,192). This has however not been investigated for LA-MRSA.

In general, more research is urgently needed to gain further information on risk factors and transmission routes of LA-MRSA. This is an essential requirement for efficient control measures to be implemented. This is of particular importance, since, in addition to its capacity to colonize humans, LA-MRSA has also been found capable to cause infections in humans. LA-MRSA has been isolated from (severe) infections of people in close contact with pigs (116,132,134,187,193,194) and poultry (130) (Table 4). There seems to be no association of LA-MRSA with certain clinical conditions, as it has been found in both invasive and skin related infections (Table 4). In addition, it is not clear whether a decreased human health condition predisposes to development of LA-MRSA infections. Further, as has been noted for animal infections with LA-MRSA, it is currently totally unclear which virulence factors are of importance in human LA-MRSA infections. However, for now, it seems that the virulence capacity and associated medical importance of LA-MRSA is much lower compared with

traditional human HA- and CA-MRSA strains. Nonetheless, future research should urgently bring more insight in these matters.

Another important aspect of LA-MRSA in humans is that, although infrequently reported and not (yet) substantiated by epidemiological data, LA-MRSA appears to be capable to transfer between humans. In the Netherlands, a six-months-old daughter of pig farmers, who presumably had not had direct contact with pigs, appeared colonized (109). In that same study, the son of a veterinarian and the nurse treating the son in the hospital to which he was admitted also appeared to be carrying LA-MRSA (109). This could create very dangerous situations, especially when personnel of medical settings carry LA-MRSA. In a Dutch hospital, a case was reported in which five healthcare workers and three patients appeared to carry LA-MRSA and two other patients were infected by LA-MRSA (133). Recently, also in a residential care facility for visually and intellectually disabled people a resident was diagnosed with dermal abscesses caused by LA-MRSA. Subsequent research revealed two other residents and three personnel members to be carrying LA-MRSA (138).

Contrary to what these cases suggest, LA-MRSA seems not to be able to spread widely in the community or in hospital settings. LA-MRSA has been known in hospitals for several years but still no large spread has been reported. Nonetheless, it is clear that LA-MRSA could complicate the MRSA control measures for hospital settings, particularly in countries that perform strict control measures, such as the Netherlands (195). Not only will the group of risk patients expand considerably, implying a higher number of screenings to be done on admission to a hospital, a higher number of screenings is also likely to lead to more people that need to be kept in isolation, implying a serious burden for hospital accommodations and healthcare means. That this problem is very relevant was shown by a recent Dutch study, in which it was found that the inclusion of the new risk group of animal farmers had lead to a 3-fold increase in the annual MRSA incidence (189).

Conclusions

MRSA in livestock should be regarded from two sides: CC398 MRSA and non-CC398 MRSA. The few reports of non-CC398 MRSA in pigs and poultry concerned MRSA of human origin. There are no indications that these animals play a role as reservoir for reinfection of humans nor that this will change in the near future. The situation in cattle is more complex. Non-CC398 MRSA is detected more frequently than in pigs and poultry, almost solely from mastitis, indicating a problem for animal health. However, the size of the problem is hard to assess. Quarter-level prevalence seems to be very low but cow-level and farm-level prevalence cannot be estimated due to a lack of consistent data.

Meat and milk are occasionally found to be contaminated with (human) non-CC398 MRSA strains. Although these food products have not yet been found to contribute to the dissemination of such strains in the community, care must be taken when drawing conclusions, due to a lack of thorough research. Until more studies have been performed normal hygiene measures and adequate preparation of the foods should suffice to contain this situation. This also counts regarding the recent detection of CC398 MRSA on various meat products.

CC398 MRSA or LA-MRSA seems primarily associated with pigs but its origin is still largely unclear. Strains of this clone have spread around the world. In livestock, besides pigs also cows and poultry are affected. However, the origin and relevance of LA-MRSA in the latter animals remains unknown to date. The implication of LA-MRSA in animal infections may pose a burden on veterinary medicine but to what extent is still unclear, as are the factors responsible for the pathogenic potential of LA-MRSA.

An essential aspect of LA-MRSA is its remarkable degree of host unspecificity, transferring also between animals and humans. Possible mechanisms explaining this host unspecificity are

yet to be revealed. In comparison to the extent of other types of MRSA, the impact on human healthcare is still small. However, the demonstration of the capacity of LA-MRSA to take-up toxin genes should urge the medical world to take measures to control transmission and spread as quickly as possible. This will require thorough research to be performed to elucidate transmission routes and risk factors.

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Table 1. Summary of SCC*mec*¹-types currently described in methicillin-resistant *S. aureus*.

Class of <i>mec</i> -complex	Type of <i>ccr</i> -complex	SCC <i>mec</i> -type	Approx. size	Reference
B	A1/B1	I	34 kbp	30
A	A2/B2	II	53 kbp	30
A	A3/B3	III	67 kbp	30
B	A2/B2	IV	21-24 kbp	31
C2	C	V	28 kbp	32
B	A4/B4	VI	24 kbp	33
C1	C	VII	27 kbp	34
A	A4/B4	VIII	32 kbp	35

¹ SCC*mec*: staphylococcal cassette chromosome *mec*

Table 2. Repeats of 25 *spa*¹-types reported to belong to CC398 MRSA² strains.

<i>spa</i> -type	Repeats	Reference
t011	08-16-----02-25-----34-----24-25	110
t034	08-16-----02-25-----02-25-34-----24-25	110
t108	08-16-----02-----34-----24-25	109
t567	08-----02-25-----24-25	109
t571	08-16-----02-25-----02-25-34-----25	116
t779	08-----	137
t898	08-16-----02-25-----02-25-34-----34-24-25	116
t943	08-16-----02-25-----25-----24-25	109
t1197	08-16-----02-25-----46-----24-25	119
t1250	08-16-----02-25-----02-25-----	131
t1254	106-16-----02-25-----34-----24-25	111
t1255	08-16-----34-----24-25	121
t1451	08-16-----02-25-----34-----25	137
t1456	08-16-----02-25-----	124
t1457	08-16-----02-25----34-02-25-34-----24-25	137
t2346	08-16-----02-25-----34-24-----24-25	134
t2383	08-16-----	138
t2970	08-16-----02-25-----34-----34-24-25	137
t3015	08-----02-25-----24-----24-25	137
t3119	08-85-----02-25-----34-----24-25	137
t4208	08-16-----02-31-----25-34-----24-25	137
t4872	08-16-----02-25-----34-24-25-34-24-25	125
t337	07-16-23-23-02-12-23----02----34-----	116

t899	07-16-23-----02-----34-----	122
t1939	07-----23-----02-----34-----	136

¹ *spa*: gene encoding *Staphylococcus* protein A

² MRSA: methicillin-resistant *Staphylococcus aureus*

Table 3. Sequence of ancestral repeats (*) and possibly derived repeats.

Repeat	Sequence
r106	GAGCAAGACAACAACAAGCCTGGT
r08*	↑ GAGGAAGACAACAACAAGCCTGGT
r07	↓ GAGGAAGACAACAACAAACCTGGT
r16*	AAAGAAGACGGCAACAAACCTGGT
r85	↓ AAAGAAGACGGCAATAAACCTGGT
r12	AAAGAAGACAACAACAAGCCTGGT
r25*	↑↑↑ AAAGAAGATGGCAACAAACCTGGT
r31	↓ AAAGAAGATGGCAACAAACCTGGC
r34*	AAAGAAGACAACAAAAAACCTGGT
r46	↓ AAACAAGACAACAAAAAACCTGGT

Table 4. Human infections caused by LA-MRSA¹.

Site of infection	Type of infection	Reference
Heart	Endocarditis	130
Wound	Diabetic foot ulcer	133
	Infection of pig bite wound	187,193
	Unspecified	119
Skin	Abscess	138,143,194
	Cellulitis	194
	Unspecified	119,132
Respiratory tract	Sinusitis	132
	Ventilator-associated pneumonia	119
Muscles	Pyomyositis	194
Unspecified	Invasive infection with multiorgan failure	132
Various	Various	134

¹ LA-MRSA: livestock-associated methicillin-resistant *Staphylococcus aureus*